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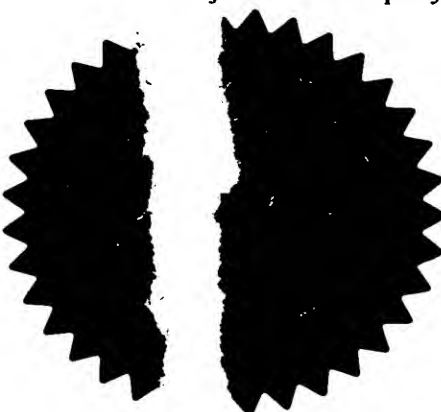
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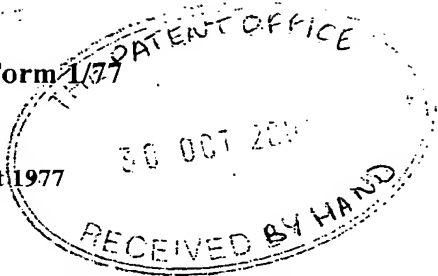
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# Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference JIM/LJ/P17532GB

2. Patent application number  
(The Patent Office will fill in) **0126026.4** **30 OCT 2001**

3. F Name of applicant (insert all surnames)  
SOCIÉTÉ DES PRODUITS NESTLÉ SA  
CASE POSTALE 353  
CH-1800 VEVEY  
SWITZERLAND

Patents ADP number (if you know it)

730762.001

If the applicant is a corporate body, give the country/state of its incorporation

SWITZERLAND

4. Title of the invention  
CHOCOLATE FLAVOUR MANIPULATION

5. Name of your agent (if you have one) ELKINGTON AND FIFE

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

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SEVENOAKS  
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TN13 1XR

Patents ADP number (if you know it)

67004

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of Filing (day/month/year)
	GB	0016173.7	30 June 2000
	GB	0118252.6	26 July 2001
	GB	0031137.3	20 DECEMBER 2000

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of Filing (day/month/year)

Ac 11/77  
4/2002

Patents Form 1/77

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer "Yes" if:

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
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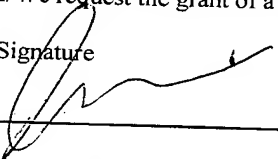
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Statement of inventorship and right to grant of a patent (Patents Form 7/77)	-
Request for preliminary examination and search (Patents Form 9/77)	1
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11.

I/We request the grant of a patent on the basis of this application.

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Date

29 October 2001

12. Name and daytime telephone number of person to contact in the United Kingdom

Mr J I MARCHANT  
01732 458881

## CHOCOLATE FLAVOUR MANIPULATION

### Field of the Invention

5 The present invention relates to processes for the manipulation of the flavour of chocolate independently of the processes, formulations and ingredient origins used in the preparation of chocolate.

### Background to the Invention

10 The process of making chocolate is described in "Industrial Chocolate Manufacture and Use", edited by S.T.Beckett, (Third Edition, 1999, Blackwell Science) the contents of which are incorporated by reference.

Chocolate is generally obtained by mixing sugar and cocoa butter with cocoa liquor or cocoa nibs, followed by refining, conching and tempering. Milk chocolate is prepared in  
15 a similar way but with the addition of milk. One traditional method of producing milk chocolate (dry process) is by mixing milk powder together with cocoa liquor or cocoa nibs, sugar, and cocoa butter, followed by refining, conching and tempering. Another traditional method of producing milk chocolate (wet process) is by condensing and drying either liquid milk or milk concentrate together with sugar with or without cocoa  
20 liquor normally under vacuum and at elevated temperatures to produce a chocolate crumb powder and then mixing the chocolate crumb powder with cocoa butter, cocoa liquor, followed by refining, conching and tempering. Optionally, the cocoa butter may be partially or totally replaced by direct cocoa butter replacements, stearines, coconut oil, palm oil, butter or any mixture thereof to give substitute chocolate materials which are  
25 generally referred to as compound, couvertures or ice cream coatings. In this invention, the term "chocolate" includes standard chocolate as well as substitute chocolate such as compound, couvertures or ice cream coatings.

Local chocolates are often unique and contain flavours that are important for the  
30 consumer and it has been known for many years to add flavours to chocolate. This is done for two reasons, firstly, modification or enhancement of the cocoa or dairy flavour,

e.g. to give a rounded smoothness to the profile or to create a creamy note, which is usually done by adding up to 0.2% of vanilla, vanillin, ethyl vanillin, etc., and secondly, to impose a different, overriding, dominant but compatible flavour, e.g. by adding orange oil, peppermint oil, strawberry, raspberry, etc.

5

However, it is well known that there are a large number of different consumer-recognisable flavour attributes associated with chocolate, other than the mere enhancement of the chocolate flavour or a different overriding, dominant flavour, which vary considerably around the world according to local consumer preferences. These flavour attributes of chocolate products are determined by variations in the process and the amounts of the normal ingredients used in chocolate manufacture, e.g. cocoa and milk. These flavour attributes may be, for example, roasted, sweet, bitter, crumb, caramel, fruity, floral, biscuit, bouquet, spicy, scented, baked, bready, cereal, popcorn, malty, astringent and praline. Such flavour attributes are well-known in the cocoa trade where they form part of the vocabulary. Consequently, local chocolates are often unique and contain flavour attributes that are important for the consumer.

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Some manufacturers produce chocolate by using chocolate ingredients and a process which only gives one flavour attribute to give a specific house flavour and the manufacturing plants are only able to produce a limited variation around this flavour. However, for a chocolate product containing chocolate and another ingredient, e.g. a chocolate biscuit or a product comprising a centre coated with chocolate, it is important to match the chocolate flavour attribute with the flavour intensity type of the other ingredient. For example, a cooked chocolate flavour attribute is desirable for a chocolate biscuit, a strong cocoa flavour attribute is required to offset a mint flavour intensity type whereas only a mild flavour attribute is required for praline which has a low intensity flavour. It would be very desirable to be able to manipulate the flavour associated with chocolate produced by a single process to obtain a flavour attribute of one's choice by adding the desired flavour attribute to a single chocolate mass irrespective of the process of preparation of the chocolate mass, the formulations and the ingredient origins. This would lead to the operation of a highly flexible chocolate plant. We have found,

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surprisingly, that these flavour attributes are not always associated with cocoa and /or milk/dairy flavours and that they may be obtained by adding non- cocoa and /or milk/dairy flavours. By "flavour attribute" in this invention, we mean a non-cocoa and/or milk/dairy consumer-recognisable flavour attribute associated with chocolate, and not a  
5 non-chocolate flavour for the mere enhancement of the chocolate flavour, e.g. by adding vanilla, or for a different overriding, dominant flavour such as peppermint.

We have found, surprisingly, that by adding the desired non- cocoa and /or milk/dairy flavour attribute to the chocolate mass, it is possible to manipulate the flavour associated  
10 with chocolate of the chocolate by adding the desired flavour attribute to a single chocolate mass irrespective of the process of preparation of the chocolate mass, the formulations and the ingredient origins. Thus a single manufacturing plant will become far more flexible and able to produce a full range of flavours.

#### 15 Summary of the Invention

According to the invention, there is provided a process for manipulating the flavour of a single mass of chocolate which comprises adding an effective amount of a non- cocoa and /or milk/dairy flavour attribute to the chocolate mass.

20 This provides a flavour attribute associated with chocolate other than chocolate flavour enhancement or an overriding, dominant flavour different to chocolate and overcomes the variations in chocolate flavour obtained in the manufacture of chocolate using different processing conditions and/or ingredients.

25

#### Detailed Description of the Invention

The chocolate mass may be a standard chocolate such as dark, milk, white chocolate or it may be a compound or ice cream coating. The milk or white chocolate mass may be a  
30 crumb chocolate or a non-crumb chocolate. Non-crumb chocolates are preferred in this process as they have less intense flavours.

The amount of flavour attribute added to the chocolate mass may be determined according to requirements and may be up to 10% by weight. For example, the amount of flavour added may be conveniently from 0.001% to 5%, preferably from 0.01% to 4%, more preferably from 0.1 to 2.5% and especially from 0.2% to 2% by weight based on the weight of the chocolate mass.

The flavour attributes may be any of the following: roasted, sweet, bitter, crumb, caramel, fruity, floral, biscuit, baked, bready, popcorn, cereal, malty, astringent or praline. The flavour attribute may be a single ingredient or a mixture of ingredients, e.g. a bottle flavour or an extracted flavour, or it may be a reaction flavour formed from a mixture of flavour precursors. For example, a crumb flavour attribute may be added to a non-crumb chocolate having a reduced flavour as compared with a normal crumb chocolate in amounts, for example, from 0.1% to 5% and may provide very desirable flavours.

Examples of non- cocoa and /or milk/dairy flavour attributes are as follows:

- 1) The flavour attribute may be a concentrate formed by adding a mixture of flavour precursors comprising
  - (A) proline, ornithine or protein hydrolysate, and
  - (B) rhamnose, fructose or fucose,to a fat-based medium and heating the mixture to about 100-140°C for about 10 –120 minutes. This flavour attribute may provide caramel and biscuit/cookie attributes to the chocolate. The amount of this flavour attribute added to the chocolate may be from 0.01-5% by weight based on the total weight of the chocolate.

Preferably, the reaction is performed at 125 °C for 30 minutes. The concentration of the flavour precursors may be about 5-250 mM, preferably about 50 mM. Preferably, the fat-based medium is anhydrous milk fat, cocoa butter, lipase hydrolysed milk fat, cocoa



liquor, butter, vegetable oils, medium chain triglycerides (MCT), triacetin, tropical fats and their fractions.

5 Preferably, the flavour precursors are proline, rhamnose and fructose. Other combinations of flavour precursors include fucose and ornithine. Milk or vegetable protein hydrolysates may be used, and are prepared from milk powder, casein, whey, soy, wheat, cotton, peanut, rice or pea protein isolates or concentrates.

Preferably, the flavour reactions may be performed by the following processes:

10 i) The fat-based media is heated and the flavour precursors (A) and (B) are dispersed in the melted fat-based medium and reacted under reflux at 125 °C. This preferably occurs under agitation. In this process, the flavour precursors are added directly in the fat-based medium without any addition of moisture.

15 ii) The flavour precursors A and B may also be dissolved in alkaline water solution, buffer at pH 5.0 - 8.0 or 0.1-50% potassium carbonate solution to form a flavour precursor solution or suspension which is then added at less than 1.5% level (w/w) to the melted fat and reacted with agitation under reflux at 125 °C. Preferably, pH of the mixture before the reaction is 8.0.

20 iii) The flavour precursors A and B may also be dissolved in alkaline water solution, buffer pH 5.0 - 8.0 or 5-50% potassium carbonate solution. The flavour precursor solution or suspension generated is added at 1.5-5% level (w/w) to the melted fat-based medium, the reaction mixture thus obtained is heated under agitation for 10-20 minutes  
25 from 100 °C to 125 °C, without closing the vessel to evaporate most of the moisture, and the remaining 10-20 minutes of the reaction is performed at 125°C under agitation and reflux. Preferably, the total reaction time is about 30 minutes. Preferably, pH of the mixture before reaction is 8.0.

30 Preferably, rhamnose and proline flavour precursors were used which were dissolved in 5% buffer (100 mM Na-phosphate pH 8.0) and added to the heated fat-based medium,

preferably anhydrous milk fat. The reaction mixture was heated for 10 minutes from 100 °C to 125 °C without closing the reactor to evaporate most of the water. The remaining 20 minutes of the reaction was performed at 125°C under reflux in the fat-based medium. This process allows maximum 4-hydroxy-2,5-dimethyl-3(2H)-furanone concentration to be obtained. Thus, the present invention has surprisingly positive results in the generation of caramel and biscuit flavour attributes using fat-based media as compared to classical aqueous systems.

Proline was used as amine flavour precursor, as proline is a precursor for caramel as well as biscuit/bread/roast type aroma volatiles. The reaction between proline and rhamnose in a fat-based medium produces a range of flavour active compounds. 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2-acetyl-1-pyrroline which are involved in caramel and biscuit/bread/roast flavour respectively, were used as chemical markers in the described invention. 4-hydroxy-2,5-dimethyl-3(2H)-furanone was the major compound in most of the reaction flavour products. The rich and balanced flavour attribute was most likely achieved by a mixture of several compounds generated in the reaction. Examples of other compounds in the reaction mixture were 3-hydroxy 2-butanone (acetoin), 1-hydroxy 2-propanone (acetol), 5-methyl furfural, 2-hydroxy 3-methyl 2-cyclopenten-1-one (corylone) and 4-acetoxy 2,5-dimethyl-3(2H)-furanone. Surprisingly, it was found that proline resulted in the highest level of 4-hydroxy-2,5-dimethyl-3(2H)-furanone accumulation in fat-based reaction systems. However, the aroma of the flavour concentrates or attributes may not be linked or limited to any of the compounds mentioned.

Addition of 5% buffer, pH 8.0 facilitated improved flavour precursor solubility and subsequent high 4-hydroxy-2,5-dimethyl-3(2H)-furanone generation and intense caramel flavour of the fat mixture. However, reduction of the aqueous phase to, for example, 1.5% can be beneficial to avoid the first stage of the reaction including moisture evaporation. It is possible to perform the reaction without any addition of aqueous solution along with the flavour precursors. In particular, this method is preferred in order to stabilize certain aroma compounds directly in the fat phase.

All fat-based reactions with rhamnose resulted in intense caramel and biscuit/cookie flavour attributes. Reactions with fructose, which is a cheaper sugar flavour precursor, resulted in substantially lower amount of 4-hydroxy-2,5-dimethyl-3(2H)-furanone. The  
5 4-hydroxy-2,5-dimethyl-3(2H)-furanone level as well as the caramel flavour could be increased by increasing the ratio of fructose to proline. Reactions in anhydrous milk fat with 50 mM proline and 100 mM fructose resulted in a further increase in 4-hydroxy-2,5-dimethyl-3(2H)-furanone level and a strong caramel and biscuit/cookie flavour. Thus, fructose and proline can also be used as flavour precursor combination in fat-based  
10 flavour reaction.

A further aspect of this invention is directed to the use of the flavour concentrates or attributes as described above in the manufacture of chocolate (including compound) using 0.01-5%, preferably 0.5% by weight of the flavour concentrate or attribute, based  
15 on the weight of the total chocolate. Preferably, the flavour concentrate or attribute is incorporated directly into the chocolate. As the flavour concentrate or attribute is generated directly in chocolate compatible ingredients, no drying or extraction is necessary before incorporation into the chocolate mass. These flavour concentrates or attributes impart caramel and/or cookie/biscuit note in the chocolate. The flavour  
20 concentrate or attribute can be added alone or in combination with other flavours.

According to a yet further aspect of this invention there is provided a chocolate product with modified flavour characteristics comprising the flavour concentrate or attribute as described above. Such chocolate products include milk, dark and white chocolate as well  
25 as compound coatings for use for example in bars or ice-cream coatings.

2) The flavour attribute may be an enzymatic hydrolysate of cocoa polysaccharides, e.g. from the cocoa shell, e.g. pectin. Such flavours may provide caramel, biscuit or toffee flavour and may be incorporated in an amount below 5% into the chocolate  
30

According to one aspect of this invention provides a cocoa shell pectin extract having a rhamnose content of approximately 5mM to approximately 100 mM. According to a further aspect this invention provides a process for the preparation of a cocoa shell pectin hydrolysate having a rhamnose content of approximately 5mM to approximately 100mM comprising the chemical and/or enzymatic hydrolysis of cocoa shell pectin. According to a still further aspect this invention provides a cocoa shell pectin hydrolysate having a rhamnose content of approximately 5mM to approximately 100mM. According to another aspect this invention provides a flavour concentrate or attribute comprising cocoa shell pectin hydrolysate according to the present invention. According to yet another aspect this invention provides the use of a cocoa shell pectin hydrolysate according to the present invention as a flavour concentrate or attribute for food.

It has surprisingly been found that the pectin from cocoa shell contains an unusually high rhamnose content of approximately 5 to 7% by weight rhamnose based on the dry weight of pectin. Thus, the present invention provides a new rich source of rhamnose which can be used to generate new flavour profiles when added to food products.

The preparation of cocoa shell pectin extracts is carried out using a standard procedure for extracting pectins.

Hydrolysis of the cocoa shell pectin extracts is carried out by chemical and/or enzymatic degradation. Preferably, the following enzymes and chemicals are used, acetic acid, hydrochloric acid, pectinolytic enzymes (polygalacturonase, rhamnogalacturonases, pectin lyase, arabinase, galactanase) in association with esterases such as rhamnogalacturonan acetylerase or with exoglycosidases such as beta-galactosidase, arabinofuranosidase and fucosidase. Mixtures of these enzymes can be found in commercial enzyme preparations produced by fermentation of microorganisms on suitable complex media.

Any suitable reaction medium for flavour generation may be used including, aqueous solutions, ethanol, propyleneglycol, glycerol, or a fat-based medium such as, anhydrous

milk fat, cocoa butter, cocoa liquor, compound fat, lipase hydrolysed milk fat, butter, vegetable oils, medium chain triglyceride, triacetin or tropical fats and their fractions.

5 The cocoa shell pectin hydrolysate can be reacted with individual free amino acids, peptides, protein hydrolysates or mixtures of amine flavour precursors.

Preferably, the preparation of flavour concentrates or attributes involves the addition of cocoa shell pectin hydrolysate to a fat-based medium, most preferably milk fat, together with proline and a phosphate buffer at pH 8.0, 125°C for approximately 60 minutes.

10 Preferably, the cocoa shell pectin hydrolysate and proline flavour precursors used are dissolved in 5% buffer (100 mM Na-phosphate pH 8.0) and are added to the heated fat-based medium, preferably anhydrous milk fat. The reaction mixture is heated for 10 minutes from 100°C to 125°C without closing the reactor to evaporate most of the water. The remaining 20 minutes of the reaction is performed at 125°C under reflux in the fat-

15 based medium. Addition of 5% buffer, pH 8.0 facilitates improved flavour precursor solubility and subsequent high 4-hydroxy-2,5-dimethyl-3(2H)-furanone generation and intense caramel flavour of the fat mixture.

The rhamnose containing cocoa shell extract may be used in different flavour reactions

20 including applications for sweet (e.g. caramel, biscuit, buttery, toffee, fruity, malty, roasted) and savory or culinary products. The flavour concentrates or attributes produced can be incorporated into foods such as confectionery products, chocolate, beverages, ice-cream, biscuits and baked products, savoury and culinary products. It may be used in chocolate manufacture including the manufacture of milk, dark and white chocolate as

25 well as compound chocolate for use in, for example, bars and ice-cream coatings.

3) A malty flavour attribute may be obtained by acid treatment of a cocoa liquor followed by a protease treatment.

30 According to one aspect of this invention there is provided a process for the production of enzymatically-treated unfermented cocoa liquor comprising acid treatment of the

cocoa liquor followed by protease treatment. Another aspect of this invention is the use of enzymatically hydrolysed cocoa liquor in generation of process flavour reactions. A still further aspect of this invention is a chocolate or compound product produced using a process flavour made using the enzymatically treated cocoa liquor.

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The acid treatment is performed as an *in-vitro* fermentation step to activate the endogenous enzyme system and to utilize the endogenous proteases in cocoa. The subsequent protease treatment is applied to obtain high degree of hydrolysis (DH) and a high level of reactive free amino acids and peptides. This treatment is used to increase the flavour precursor pool during chocolate flavour reactions. Cocoa hydrolysates produced in accordance with this process are used as an ingredient in process flavour reactions. The cocoa liquor hydrolysates may be used alone or together with other ingredients or flavour precursors, such as amino acids, peptides or sugars, as a source of amine flavour precursors in process flavour reactions.

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The cocoa liquor may be fully or partially defatted prior to use. Such use of fully or partially defatted cocoa liquor enables flexible use of different cocoa materials as a base ingredient in process flavour reaction. Preferably, the acid treatment involves decreasing the pH of the unfermented cocoa liquor to approximately pH 2 to 5, most preferably to pH 4. Preferably, acetic acid, citric acid or phosphoric acid is used at a concentration of from about 0.01 to about 1.0 M. Protease treatment involves the incubation of the unfermented cocoa liquor mixture with an endoprotease and/or exoprotease. Preferably, from 0.1% to 5% protease based on dry weight of cocoa is used. Most preferably, the cocoa is incubated with 0.1 M acetic acid at 50 °C for 6 hours, followed by treatment with 2% protease, based on dry weight of cocoa, for 18 hours at 50 °C. Treatment times for both the acid and protease treatment are preferably from 1 to 48 hours.

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The treated cocoa liquor can be used in process flavour reactions. In the flavour reactions generation of a balanced cocoa flavour is not the only criterion. The introduction of other attributes, for example caramel, biscuit, fruity and malt are also of interest. Thus, another

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objective was to produce a cocoa ingredient with a maximum degree of hydrolysis for use in chocolate flavour reactions.

5 The process flavour attributes made using the treated cocoa liquor can be used in the manufacture of standard chocolate, compound chocolate, ice-cream coatings and in other food products, desserts and drinks.

10 4) A caramel flavour attribute may be provided by the reaction of skimmed milk powder in a fat such as milk fat, cocoa butter, cocoa butter substitute, cocoa liquor, vegetable fats or combinations of fats at an elevated temperature. The amount of skimmed milk powder may be from 0.5% to 15% by weight based on the weight of the mixture. The temperature of the reaction may be from 100°C to 150°C and preferably from 115°C to 135°C. The duration of the reaction may be from 15 minutes to 2 hours and preferably from 30 to 90 minutes. The caramel flavour attribute may be incorporated into chocolate from which the natural flavour has been reduced or removed in an amount from 0.1% to 15 5% and preferably from 0.5% to 2% by weight based on the weight of the chocolate.

The present invention also provides a process for manipulating the flavour of a single mass of chocolate which comprises adding an effective amount of a non-cocoa and/or 20 milk/dairy flavour attribute to the chocolate mass independently of the chocolate mass processes, formulations and ingredient origins.

The flavour attribute may be added to any of the ingredients of the chocolate mass and at any stage of the chocolate-making process before tempering. A major advantage of the 25 invention is the production of chocolate and compound coatings with a particular flavour independently of its assets, processes, formulations and ingredient origins.

The invention permits the development of chocolate products with a global flavour, optimization of asset utilization, cost reduction, recipe flexibility and development of 30 products with particular flavours that are preferred by the local consumers.

The following Examples further illustrate the present invention.

#### Example 1

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The flavour reactions were performed in a round bottom 3-necked stirred reactor with temperature control. Anhydrous milk fat (80g) was melted in the reactor and heated to 125°C. The flavour precursors, rhamnose (50 mM) and proline (50 mM), were added directly with the milk fat or added separately when the temperature of the milk fat had reached 125 °C. Rhamnose and proline were reacted in the milk fat for 60 min at 125 °C under reflux. The reacted material was allowed to cool to room temperature and stored at 4°C or room temperature. The flavour attributess were incorporated at 0.5% level of addition into a chocolate mass.

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#### 15 Incorporation into compound:

One gram of the reaction flavour was added to 199 grams of completely melted compound mass, thoroughly mixed by hand, and molded into 20 gram bars. Following cooling, bars were demolded and allowed to equilibrate to room temperature for at least 4 hours. The samples were allowed to equilibrate at 15 °C for 1-30 days before tasting.

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#### Incorporation into white and milk chocolate:

One gram of the reaction flavour was added to 199 grams of completely melted chocolate mass and mixed manually at 50 °C to homogenize the sample, followed by precrystallization and molding into 5 g bars. Following cooling, bars were demolded and allowed to equilibrate to room temperature for at least 4 hours. The samples were allowed to equilibrate at 15 °C for 1-30 days before tasting.

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The chocolate samples were evaluated by blind tasting with 6-9 trained panellists. All samples were compared to the reference chocolate mass without any incorporation. Incorporation of the fat-based flavour resulted in strong enhancement of attributes such as caramel, biscuit, and cookie in the chocolate mass.

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Volatile analysis of the flavour concentrates was carried out using solid-phase micro-extraction (SPME) combined with gas chromatography-mass spectrometry. The generation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2-acetyl-1-pyrroline was selected as chemical markers for caramel and biscuit flavour development, respectively (Figure 4). 4-hydroxy-2,5-dimethyl-3(2H)-furanone was the major peak in the chromatogram. 2-acetyl-1-pyrroline was also identified in the reaction mixture. It is clearly shown in Figure 1 that reactions in fat-systems facilitates high accumulation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone. It is furthermore shown that proline as flavour precursor facilitates 4-hydroxy-2,5-dimethyl-3(2H)-furanone accumulation as compared to arginine (Figure 2).

#### Example 2

A flavour reaction was performed according to example 1, except that 1.5% of alkaline water (Stock solution: 4 drops of 50% NaOH in 20 ml water) was added along with the rhamnose and proline. This approach facilitated the reactions at basic pH and increases the solubility of the precursors. Incorporation of 0.5% of the fat-based flavour attribute into chocolate, as described in example 1, resulted in attributes such as caramel, biscuit and cookie. Strongest caramel flavour was achieved after 30 min of reaction.

#### Example 3

A flavour reaction was performed according to example 1, except that the rhamnose and proline flavour precursors were dissolved in 100 mM Na-phosphate buffer, pH 8.0 and added when the milk fat had reached a temperature of 100 °C. This approach facilitated the reactions at basic pH and increases the solubility of the precursors. The mixture was heated without closing the reactor until most of the moisture had evaporated and the product temperature had reached 125 °C. The reaction was continued under reflux at 125 °C. Total reaction time was 30-60 min. Incorporation of 0.5% of the fat-based flavour attribute into chocolate, as described in example 1, resulted in attributes such as caramel,

biscuit and cookie. Strongest caramel flavour was achieved after 30 min of reaction. The strong caramel flavour was correlated to high 4-hydroxy-2,5-dimethyl-3(2H)-furanone level.

#### Example 4

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A flavour reaction was performed according to example 1, except that the flavour precursors were 50 mM fructose and 50 mM proline. Incorporation of 0.5% of the fat-based flavour into chocolate resulted in attributes such as caramel and biscuit. Strongest biscuit flavour was achieved after 60 min of reaction. Fructose/proline reaction in anhydrous milk fat resulted in the highest accumulation of 2-acetyl-1-pyrroline. The level of 4-hydroxy-2,5-dimethyl-3(2H)-furanone was lower as compared to reactions with rhamnose.

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#### Example 5

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A flavour reaction was performed according to example 4, except that the flavour precursors were 100 mM fructose and 50 mM proline. Incorporation of 0.5% of the fat-based flavour attribute into chocolate resulted in attributes such as caramel, cookie and biscuit. The caramel attribute was enhanced by increasing the fructose concentration from 50 to 100 mM.

20

#### Example 6

A flavour reaction was performed according to example 4, except that the flavour precursors were 1% fructose and 1% casein hydrolysate. The casein hydrolysate was prepared by standard techniques using Flavourzyme 1000 L (fungal protease/peptidase mixture from Novo Nordisk, Denmark). Hydrolysis was performed at 50°C using 1% enzyme by weight of protein content to achieve approximately 50% degree of hydrolysis. Incorporation of 0.5% of the fat-based flavour attribute into chocolate resulted in an increase in the caramel attribute.

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#### Example 7

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Ornithine, which is a well known precursor of biscuit/bread/baked impact compounds was also reacted in the milk fat system. A flavour reaction was performed according to example 2, except that the flavour precursors were 50 mM rhamnose and 50 mM ornithine. Incorporation of 1.0 % of the fat-based flavour attribute into chocolate resulted in an increase in the caramel attribute. Although a flavour impact was achieved with  
10 ornithine, it was less pronounced than with proline.

#### Example 8

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A flavour reaction was performed according to example 1, except that the flavour precursors were 50 mM fucose and 50 mM proline. Incorporation of 1.0 % of the fat-based flavour attribute into chocolate resulted in an increase in the caramel and biscuit attributes.

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#### Example 9

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Cocoa butter can also be used as reaction medium. A flavour reaction was performed according to example 1 except that the reaction medium was cocoa butter. The flavour precursors were 50 mM rhamnose and 50 mM proline. Incorporation of 1.0 % of the fat-based flavour attribute into chocolate resulted in an increase in the caramel and biscuit attributes. Again, the combination of proline/rhamnose in presence of 5% buffer, pH 8.0 was proven optimal to obtain high 4-hydroxy-2,5-dimethyl-3(2H)-furanone concentration. Although the 4-hydroxy-2,5-dimethyl-3(2H)-furanone concentration was high in these samples, the panellists perceived lower caramel as compared to chocolate  
30 samples prepared with reaction flavours in milk fat. Thus, milk fat seems to contribute to the flavour when incorporated in the final chocolate.

#### Example 10

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A flavour reaction was performed according to example 3 except that the reaction medium was cocoa liquor. The flavour precursors were 50 mM rhamnose and 50 mM proline. Incorporation of 1.0 % of the fat-based flavour attribute into chocolate resulted in an increase in the caramel and biscuit attributes.

10

#### Example 11

15 A flavour reaction was performed according to example 5 except that the reaction medium was lipase hydrolysed milk fat. Hydrolysis was performed with an immobilized lipase, Lipozyme RM IM from Novo Nordisk, Denmark. The flavour precursors were 50 mM proline and 100 mM fructose. Incorporation of 0.2 % of the fat-based flavour attribute into chocolate resulted in an increase in the caramel, biscuit, and cheesy attributes

#### 20 Example 12: Enzyme-treated 1 M KOH-soluble extract from cocoa shell

Preparation of 1M KOH-soluble extract:

25 10g of cocoa shell was extracted in 200 ml of 1 M KOH containing 20 mM NaBH<sub>4</sub> for 4 hours at room temperature. The suspension was centrifuged and the supernatant neutralised with acetic acid, dialysed and then freeze dried. The composition of the extracted polysaccharide is shown in table 1.

Table 1 : Composition of 1 M KOH-soluble cocoa shell fraction

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA*	Total
µg/mg	33.2	1.6	13.9	22.1	25.3	93.4	42.5	290	522
Mole%	7.1	0.3	3.3	5.2	4.7	17.5	7.9	54.0	-

Enzymatic treatment:

An aliquot of the extract obtained (1g in 50 ml of distilled water) was hydrolysed with 0.25 ml of Viscozyme L, a multi-enzyme complex produced from *Aspergillus aculeatus* (Novozymes A/S, Denmark), at 40°C for 16 hours with stirring. The pH during incubation was 4.9. The mixture was then freeze dried. Determination of free rhamnose in the final product indicated that 18.2 % of rhamnose was liberated from the 1 M KOH-soluble cocoa shell fraction by enzymatic treatment. The results are shown in table 2.

Table 2:

	Experimental	Rha (µg)	Powder (mg)	Rha(%)
Total	2 M TFA – 120°C – 2 h	155	3.1	5.0
Liberated by Viscozyme	Soluble in 70% EtOH	246	27	0.91

Yield (%) 18.2

Example 13: Production of acid hydrolysate from Na<sub>2</sub>CO<sub>3</sub>-soluble pectin of cocoa shell

11.5 g of sodium carbonate extracted pectin was hydrolysed in 575ml 2M HCl by autoclaving at 120°C for 1h. The hydrolysate was filtered to remove black sediment and then dried. When the volume was reduced to about 50ml acetic acid was added and the solution dried down and held under vacuum for 3 days over NaOH pellets to remove residual acid. Water was added and the solution was evaporated to dryness and the flask was dried again over P<sub>2</sub>O<sub>5</sub> and NaOH pellets. The drying down process was repeated twice.

The dried material weighed 9.6 g. It was dissolved in 100 ml water and half was adjusted to pH 5.8 with 2-3 ml of 2.5 M NH<sub>4</sub>OH and filtered through glass fibre paper to remove undissolved residue and passed through a column of QAE Sephadex which had been

converted to the formate form. The hydrolysate in the column was eluted with 350ml water. The neutral fraction, which was not retained on the column and the acidic fraction (recovered by eluting the column with 350 ml 10% formic acid) were each dried down on the rotovap and the latter fraction put over NaOH and P<sub>2</sub>O<sub>5</sub> under vacuum overnight. Table 3 shows the composition of the hydrolysate fractions prepared.

	Wt of fractions:
Original Na <sub>2</sub> CO <sub>3</sub> -sol pectin	11.5 g
Insoluble fraction after hydrolysis	1.4 g
Total soluble acid hydrolysate	8.4 g
Neutral fraction (from half the hydrolysate)	1.4 g
Acidic fraction (from half the hydrolysate)	2.4 g

Table 3 : Composition of hydrolysate fractions (µg/mg)

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA	Total
Total	34.0	0.3	3.2	1.0	9.7	33.9	10.1	210.4	303
Neutral	68.9	0.8	4.6	1.6	17.2	59.6	18.3	189.0	358
Acid	2.9	4.3	0.7	0.9	1.3	4.6	2.8	351.0	369

Example 14: Use of hydrolysates in the preparation of reaction flavours and incorporation into white chocolate

The hydrolysates prepared in accordance with examples 12 and 13, including the total hydrolysate and the hydrolysate purified by cation exchange were incorporated into a reaction flavour comprising 8.5g milk fat, 1g shell hydrolysate (5mM free rhamnose), 50mM Proline, 5% phosphate buffer at pH 8.0, 125°C for 60 minutes. The reaction flavour thus obtained was then incorporated into white chocolate at a level of 1.0-1.5% by weight based on the total mentioned chocolate in accordance with the aforementioned recipe and subjected to sensory evaluation. Sensory evaluation of the white

chocolate produced using the hydrolysates of examples 12 and 13 showed an increased caramel flavour.

5 Example 15: Use of hydrolysates in the preparation of reaction flavours and incorporation into milk chocolate

The total hydrolysate and the hydrolysate prepared by cation exchange prepared in accordance with example 2 were incorporated into reaction flavour attributes A and B comprising

- 10 A) 8.5g milk fat, 2.28g total shell hydrolysate (50mM free rhamnose), 50mM Proline, 5% phosphate buffer at pH 8.0, 125°C for 60 minutes; and  
B) 10.5g milk fat, 1.11g shell hydrolysate purified by cation exchange (50mM free rhamnose), 50mM Proline, 5% phosphate buffer at pH 8.0, 125°C for 60 minutes.

15 Reaction flavour attributes A and B were then incorporated into milk chocolate at a level of 3% by weight based on the total mentioned chocolate in accordance with the aforementioned recipe and subjected to sensory evaluation. Sensory evaluation of both milk chocolates A and B prepared showed an increased caramel flavour attribute.

20 Example 16: Use of enzymatically treated cocoa liquor in reaction flavours :

A reference cocoa reaction flavour was prepared by reacting 0.8% Leu, 1.45% Phe, 0.8% Val, 1.5% Fructose, 1.5% water (4 drops of NaOH in 20ml water) and 94% propylene glycol at 125°C for 60 min under reflux. Reaction flavours, prepared with cocoa  
25 hydrolysates were generated by replacing the amino acids with 1% lyophilised hydrolysate. Tasting was performed on a 0.1% solution in 1% sucrose. The reaction flavours produced with cocoa hydrolysates were tasted and compared against the reference.

The following enzyme treatments were investigated: 24 h and 6 h *in-vitro* fermentation/Flavourzyme treatment, and 24 h and 6 h *in-vitro* fermentation (only endogenous enzyme system).

5      Reaction flavour attributes prepared with enzyme-treated liquor exhibited stronger cocoa flavour compared to a control prepared with untreated unfermented/unroasted cocoa liquor. In particular, the reaction flavour attributes produced with liquor that was treated by only *in-vitro* fermentation seemed strongest in cocoa flavour.

10      The reaction flavour attributes generated with 1% cocoa liquor hydrolysates in PG do not contain similar amount of reacting amino groups as in the amino acid reference. The reference is prepared with 0.8% leu, 1.45% phe, 0.8% val, 1.5% fructose, whereas the reaction mixtures with cocoa hydrolysates contains substantially lower level of free amino groups (10-15% protein, DH 10-30). Thus, the amount of reactive amine flavour  
15      precursors can be increased substantially by increasing the proportion of hydrolysed cocoa liquor.

The cocoa hydrolysates, described in this example, can be used as a base ingredient in chocolate process flavour reactions to introduce a base or specific note for a full body  
20      chocolate/cocoa flavour concentrate by adding 2% by weight of the concentrate to a melted chocolate mass.

#### Example 17

25      10 parts of skimmed milk, 45 parts of cocoa butter and 45 parts of cocoa liquor are blended and reacted at 125°C for 60 minutes to give a caramel reaction flavour attribute.

Incorporation of 1.0 % of the above caramel flavour attribute into chocolate resulted in  
30      an increase in the caramel attribute.

#### Example 18



A milk chocolate compound coating was first prepared according to the following general composition:

Component	Weight Percentage
Sucrose	50.00
Non fat dry milk (NFDM)	13.78
Cocoa	6.00
Vegetable Fat	30.00
Vanillin	0.02
Lecithin	0.2

- 5 One flavor compound was added to the milk chocolate compound coating and evaluated by a descriptive panel trained in tasting chocolate and cocoa:

Compound	Concentration (ppm)	
	2174-112a	2174-112b
2,5-dimethyl-4-hydroxy-3(2H)-furanone	0	10

- 10 Results showed that addition of 2,5-dimethyl-4-hydroxy-3(2H)-furanone at 10 ppm to the compound coating increased significantly the caramelized sugar (p-value = 0.0112) and cocoa (p-value = 0.0075) attributes relative to the unflavored control.

#### Example 19

- 15 Volatile cocoa aroma compounds from a cocoa nib grinder were captured and condensed using technology described in United States Patent No. 6090427. A portion of this residue was added to a chocolate compound coating similar to the one described in example 18.

Compound	Concentration (%)	
	2176-17	2174-53c
Grinder Gas Residue	0	0.15

- 20 Results showed that addition of the grinder gas residue to the compound coating increased significantly the Fruity attribute (p-value = 0.0016) relative to the unflavored control.

#### 25 Example 20

One flavor compound was added to a milk chocolate compound coating similar to the one described in Example 18:

Compound	Concentration (ppm)	
	Unflavored	2150-36C
Phenethyl alcohol	0	20

30

When evaluated blind against the unflavored control by panelist experience in chocolate tasting, the sample with added phenethyl alcohol was considered to be higher in a floral (rose-like) attribute by all panelists.

5

CLAIMS:

1. A process for manipulating the flavour of a single mass of chocolate which comprises adding an effective amount of a non-cocoa/dairy flavour attribute to the chocolate mass.
2. A process according to claim 1 wherein the flavour attribute provides any of the following attributes: roasted, sweet, bitter, crumb, caramel, fruity, floral, biscuit, baked, bread, popcorn, cereal, malty, astringent or praline.
3. A process according to claim 1 wherein the flavour attribute is a single ingredient or a mixture of ingredients or it is a reaction flavour attribute formed from a mixture of flavour precursors.
4. A process according to claim 1 wherein the amount of flavour attribute added to the chocolate mass is from 0.001% to 15% by weight based on the weight of the chocolate mass.
5. A process according to claim 1 wherein the flavour attribute is a concentrate formed by adding a mixture of flavour precursors comprising
  - (A) proline, ornithine or protein hydrolysate, and
  - (B) rhamnose, fructose or fucose,to a fat-based medium and heating the mixture to about 100-140°C for about 10 -120 minutes.
6. A process according to claim 1 wherein the flavour attribute is a Maillard reaction between defined mixtures of amino acids and sugars in chocolate compatible fat systems, roasted using cocoa liquor technology in the presence or absence of water.
7. A process according to claim 1 wherein the flavour attribute is an enzymatic hydrolysate of cocoa polysaccharides.

8. A process according to claim 1 wherein the flavour is a malty crumb flavour obtained by acid treatment of a cocoa liquor followed by a protease treatment.
- 5 9. A process according to claim 2 wherein the crumb flavour attribute is added to a non-crumb chocolate mass in an amount of from 0.1% to 5%.
- 10 10. A process according to claim 2 wherein the flavour attribute is a caramel flavour attribute provided by the reaction of skimmed milk powder in a fat system at an elevated temperature.
11. A process for manipulating the flavour of a single mass of chocolate which comprises adding an effective amount of a non-cocoa/dairy flavour to the chocolate mass independently of the chocolate mass processes, formulations and ingredient origins.

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ABSTRACT

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CHOCOLATE FLAVOUR MANIPULATION

10 A process for manipulating the flavour of a single mass of chocolate which comprises  
adding an effective amount of a non-cocoa and/or milk/dairy flavour attribute to the  
chocolate mass wherein the flavour provides any of the following attributes: roasted,  
sweet, bitter, crumb, caramel, fruity, floral, biscuit, baked, bready, cereal, malty,  
astringent or praline.

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